Tropinin T Release in Hemodialysis Patients

To the Editor:

We read with interest the recent article by Diris et al, investigating the reason for elevated serum concentrations of cardiac troponin T (cTnT) in hemodialysis patients. The authors found variable amounts of different cTnT fragments in the blood of hemodialysis patients, ranging in size from 8 to 25 kDa. In contrast, intact cTnT (39 kDa) was found only in the cTnT-spiked, pooled serum from healthy subjects. Median concentrations of cTnT increased significantly with longer duration of hemodialysis. From these findings, the authors speculated that in vivo fragmentation of cTnT occurs only in hemodialysis patients and that impaired renal function causes the unexplained accumulation of these fragments in blood. The authors further hypothesized that elevated cTnT is not the result of a cardiac troponin T release due to impaired hemodynamics or underlying cardiac disease but is rather the result of accumulating cTnT fragments from small amounts of troponin released continuously from physiological cardiomyocyte decay.

We believe that these conclusions are not justified for several reasons, as follows.

First, Western blot is extremely sensitive to accumulation and nonspecific binding of antibodies on the electrophoresis gel. However, information on the specificity of the monoclonal mouse antibody prepared from clone 4C5 (Fortron) has not been provided. Thus, it may be that not cardiac but skeletal troponin T is identified by the antibodies. It may even be that it is not cTnT fragments at all that are detected. Properly designed experimental investigation would have required amino acid sequencing of the protein bands on Western blot. Such an analysis is a standard procedure in proteomic research and definitely required before an interpretation is allowed on the protein nature of antibody staining in Western blot.

Second, we agree with the authors that renal dysfunction impairs the clearance of intact cTnT from blood, resulting in an amplification of the marker concentration in blood. We also agree that there might be a continuous release of cardiac constituents in healthy persons, but this is not yet measurable with current assay formats.

However, we disagree with the hypothesis that elevated cTnT in hemodialysis patients is due to the accumulation of a physiological troponin release after microloss of myocytes. Patients with end-stage renal disease are not healthy subjects by definition.

A consistent body of evidence from large observational trials has established beyond doubt that cTnT concentrations are inversely related to prognosis in patients with end-stage renal disease. Thus, increasing concentrations of cTnT with duration of hemodialysis support the concept of progressive cardiac or coronary disease and the prevalence of complex lesion morphology and multiple comorbid conditions such as diabetes, hypertension, congestive heart failure, a history of previous myocardial infarction, or a history of previous coronary artery bypass grafting rather than the concept of a time-dependent accumulation of cTnT fragments. How could the authors otherwise explain the increased mortality and cardiac morbidity associated with cTnT at the lowest detectable range? The authors seemingly ignore the important clinical information and may mislead the readers by unconfirmed and inaccurately conducted Western blot analyses. This may prevent responsible physicians from reacting to elevated cTnT levels in hemodialysis patients. Also, the reasoning that minor elevations of cTnT in the presence of normal CK-MB mass “are unlikely due to recent myocardial ischemia” is in contrast with accumulated scientific evidence from large clinical trials.

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Prof Katus invented the troponin T assay and holds a patent jointly with Roche Diagnostics, Germany.


Response

We thank the authors for their comments on our article about troponin T (TnT) fragments in hemodialysis patients, but we regret that they question the nature of the electrophoresis bands that we identified as cardiac TnT fragments. We have performed numerous Western blot control experiments using not only the 4C5 (Fortron) antibody, but also anti-TnT antibodies with documented cross-reactivity with skeletal TnT of <0.01% (Research Diagnostics Inc). In our paper, we mentioned that negative control sera do not show any bands, whereas identical samples spiked with purified human cardiac TnT initially show only 1 band at 39 kDa, excluding any sample matrix effect. Unpublished experiments showed that after in vitro incubation at 37°C, this intact TnT disappears, whereas smaller fragments (of comparable molecular weight as found in patients) appear. Furthermore, fragmentation patterns found in patients with acute myocardial infarction show time-dependent changes, including absence of TnT bands in early samples after onset of symptoms when TnT concentrations are still <0.01 µg/L. Taken together, these findings confirmed and validated our Western blot findings. We finally chose to submit a Western blot using the 4C5 antibody because it appeared to bind with high affinity to an epitope in the center of the TnT molecule, thereby displaying most of the fragments already found with the other antibodies with epitopes toward the C- and N-terminal sites of the molecule.

As mentioned in our article, we do not question the predictive value of cardiac TnT. We agree that most dialysis patients “are not healthy subjects,” and we certainly do not exclude the possibility of TnT elevations due to cardiac or coronary disease. However, even in the absence of smoking, hypertension, and diabetes, and with a normal ECG and no history of cardiac disease, dialysis patients are found with detectable TnT concentrations and circulating TnT fragments leaving physicians clueless. In our paper, we tried to provide the physicians confronted with these patients with information about an underlying phenomenon that could contribute to these minor elevations. As much as it is unwanted to neglect truly increased troponin values, it is equally harmful to be uncritical towards every increase in serum TnT in dialysis patients, as unnecessary diagnostic or therapeutic measures can be avoided by judging the chance of finding significant coronary disease in a patient. We believe that with this additional information and complete status of the patient, the physician is able to respond appropriately.

We have preliminary data regarding possible differences in fragmentation patterns between acute myocardial infarction patients.
with and those without decreased renal function. Final proof about the relation between Western blot and the TnT assay will be obtained when the same antibodies can be used. A cooperative search for distinctive TnT fragments may provide the answer as to whether, in a specific patient, minor elevations are caused by decreased renal clearance or by increased cardiac damage.

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